# DESIGN FORMULATION DEVELOPMENT AND EVALUATION OF NOVEL NANOEMULSION FOR OCULAR DRUG DELIVERY OF LEVOFLOXACIN

SYNOPSIS OF THE THESIS

*Submitted by*

*SHIVALIKA*

(Reg. No. 17RCPPHP002)

Under supervision of

Dr. AMIT CHAUDHARY (GUIDE)

Dr. BHUPENDRA TOMAR (CO-GUIDE)

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF PHARMACY

FACULTY OF PHARMACY

ABHILASHI UNIVERSITY

**Abhilashi University Mandi**
**Chailchowk (Chachiot) Distt. Mandi (H.P.)**
**Ph: 01907-250408, 9418006520, 9816700520, 9816005139**
**H.O. Ner Chowk, Tehsil Sadar, Distt. Mandi H.P. 175008**
**Ph: 01905-248194, 248198, 248191, 243328, 248539**

## DECLARATION

I hereby declare that the dissertation synopsis entitled "**Design Formulation Development and Evaluation of Novel Nanoemulsion for Ocular Drug Delivery of Levofloxacin**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

**Place:** Chailchowk

**Date:** 31-7-19

Shivalika 31/7/19

**SHIVALIKA**

*(Reg. No. 17RCPHP002)*

**Forwarded by:**

*CO-SUPERVISOR*

**Place:** Ner-chowk.

**Date:** 30/07/2019.

Tomar

**Dr. Bhupendra Tomar**

**Forwarded by:**

*SUPERVISOR*

**Place:** Chail-chowk.

**Date:** 31/07/2019.

Amit Chy 04/07/2019

**Dr. (Prof.) Amit Chaudhary**

***Dean***

*Dept. of Pharmaceutics, Abhilashi University, Chail Chowk, Mandi*

# TABLE OF CONTENTS


| Sr. No. | PARTICULARS | PAGE No. |
|---|---|---|
| 1. | INTRODUCTION | 1-12 |
| 2. | LITERATURE REVIEW | 13-20 |
| 3. | AIM AND OBJECTIVE | 21 |
| 4. | PROPOSED RESEARCH METHODOLOGY | 22-30 |
| 5. | REFERENCES | 31-33 |

## INTRODUCTION

Ocular drug administration: This medication technique helps the administered ophthalmic drug by targeted area of site of ocular passage it's in maximum retained concentration providing relief. The complex function, anatomy & physiology of the eye, like i.e., Cornea's impermeability, inner and outer blood-retinal barrier, nasolacrimal drainage system, limitations of non-corneal structures, pose a challenge to ocular drug administration as; it makes the bioavailability of ophthalmic drug rare by diluting the concentration of administered drug. Basis on above feature of eye, significant advances and research are been carried to optimize the ophthalmic drug remittance negating the ocular barriers [(1)].

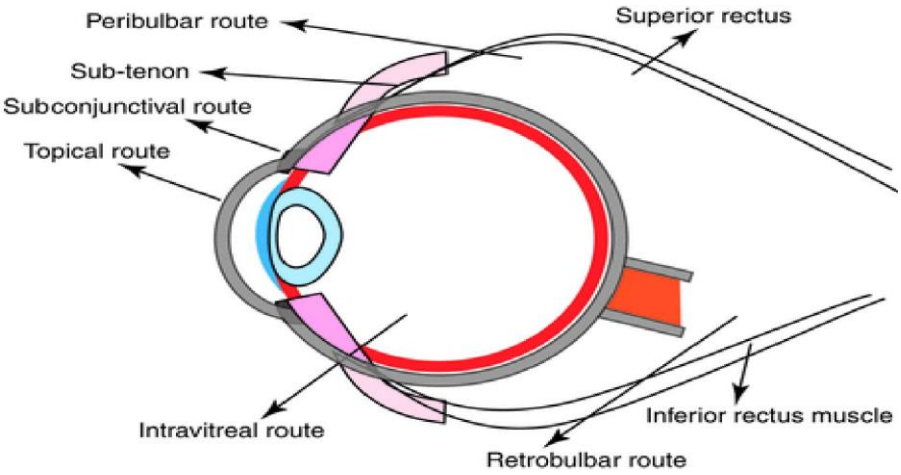



**Fig: 1: Ocular Drug Administration Routes.**

### 2. DOSAGE FORMS APPLIED TO THE EYE

Ocular tissue's receptivity to irritation, sterilization are the critical considerations to all ophthalmic dosage forms

**Ocular solution:**

It is a sterile and aqueous form of dosage, administered through ocular passage and may contain excipients providing stability to the solution in terms of pH, viscosity, osmotic pressure, alongside the preservatives (if to be used for long).

**Ocular Topical liquid:** Topical dosage form (drop) is an aqueous solution considered to be safe, effective and economical mode of drug administration. We use various additives, i.e.

cyclodextrins, permeation enhancers, viscosity enhancers, which benefits by increasing the permeation, ocular bioavailability and effective contact period [(2)].

**Ocular Suspensions:**

Ocular suspensions are the heterogeneous mixture formed by suspending agent and qualified solvent medium. The micro size of drug powder saves eye from irritation and scratches to the cornea.

**Ocular ointment:**

Ophthalmic ointment are the semisolid bases, having a melting point close to body temperature, when applied, it melts and diffuses its active ingredients in the eye, giving a greater ocular contact period along with blurred vision for tiny period of time.

**Ocular Emulsions**

Ocular emulsion's formation is a spontaneous process, where we mix the active ingredients in oil phase, which is then mixed with water, after adding suitable dispersing agents in the mixture. Emulsions are a stable system of two unmixable fluids i.e., oil in water and water in oil dispersiontype of dosage forms stabilized by surfactant or co-surfactant. Ocular emulsions are naturally degradable and can be sterilized.

**Ocular Strips**

Ophthalmic strips individually packed and sterilized. Various drugs used to impregnate the strip are (i) fluorescein sodium (ii) is amine green: (iii) rose Bengal

**Ocular Injections**

It is a parental formulation used as the suitable physical form.

**Ocular Inserts**

Made of flexible polymer, Ophthalmic inserts are non-allergic, sterile, insoluble to tears and bio-inert are put at white part of eye Ocular inserts are not used for a longer time period as they lack preservatives. These inserts have increased time of contact with drugs and eye tissues, which enhances bioavailability with a sustained action of drug. This drug form contains solidity to its form leading to reluctance from patient, difficult to insert at right place & expensive.

**Ocular Contact Lenses**

Ocular contact lens is narrow, circular & bend piece of pliant directly put upon the eye surface. Using methods like simple soaking, imprinting and colloidal nanoparticles, drug is incorporated

and contacts lenses helps in increasing the residence time of the drugs, treating exterior eye disorders. Sustaining the medicament liberate in eternity and integrate enough medicament quantity over optic grid are some of the challenges this technique poses. Conventional hydrogel soft contact lenses possess an capability to time period for lens is submerged in the drug loading solution, allows it to absorb and release some drugs into the post lens lacrimal fluid.

**Ocular Implants**

Implants helps us to extend drug in ocular fluids and posterior eye tissues, implants are (i) biodegradable (ii) non-biodegradable & physically they can be classified as solids, semi solids.

**Ocular Medication implement mixer compound:**

Ocular Medication implement mixer compound is that type of compound which has facilitator for the specific target area. Such as verteporfin for injection, is one such FDA approved combination product, associated with nonthermal red light activation [(3)].

**Modern ocular drug delivery Formulation:** The various types of modern ocular drug delivery formulation are liposomes, microspheres, microcapsules, nanoparticles or nano capsules, microemulsion, colloidal formulation, Nanosuspension, Niosome, Dendrimers, Hydrogel, etc. they may be applied in liquid form, just like eye-drop solutions, these formulations have low viscosity. Thus, they avoid the discomfort often associated with viscous gels and ointments but still provide a reservoir from which the drug can be delivered slowly.

**Liposomes** It may increase the ocular bioavailability of certain drugs by increasing the association of the drug with the cornea by means of an increased lipophilic liposomal bilayer interaction with the corneal epithelium Microparticles and nanoparticles are colloidal drug carriers in the micrometer and sub-micrometer range.

**Microspheres/Nanoparticles,** Nanoparticles are not only comfortable but give prolonged release of drugs. The drugs are bound to small particles which are then dispensed in aqueous vehicles. They are akin to colloidal solution. Nanoparticles of poly butyl cyanoacrylate without any drug insaline have been used for human beings as drug carriers. Similarly other substances are being evolved for this purpose. Some polymeric colloidal dispersions and film forming agents termed ‘pseudo plastic’ have also been used for sustained action in topical ophthalmic products.

**Ocular colloidal formulation** is formulations of such as liposome’s, nanoparticles, microemulsion, and nanoemulsion. It is applied and evaluated for ocular system. But limited use of colloidal formulation because of not well balanced throughout purification.

**Microemulsion** It is small droplets size and transparent. it is incorporated with surfactants and cosurfactants. If high number of surfactants and cosurfactants used in microemulsion it produces toxicity.

**Nanosuspension** Nano suspension is alternate nanometer of silver protein (silica gel), it is a mixture of solution which is composed of imperfectly aqua dissolvable medication, and it is diffuse during till proper disbandment form hold with surface active agent. Mainly, it contains mixture solution bearer such as chemical compound (silicon) with cohesive substance. That is motionless.

**Niosome** It may compose of two layers of follicle consist of nonpolar surface-active agent or also encapsulates together lipotropic or deliquescent mixture. Their capability to liberate the medicament, unconventional hydrogen ion concentration, enhances ophthalmic bioavailability. The structures are made up of by adding cholesterol with subsequent hydrated aqueous media to a mixture of alkyl class nonionic surfactant or dialkyl class polyglycerol ether. Niosome can be unilamellar or multilamellar on the basis of their preparation method.

**Dendrimers** These are the organic compound. It is flake like structure seen in the microscope. Molecular weight and size of the dendrimer play in an important role in choosing in the exterior functional group while designing a delivery system.

**Hydrogels**, Hydrogels are 3-D, deliquescent polyamide grid. In case of pulp (cellulose) interactivity in lacking affinity of water component in rising temperature, which can also be achieved by dissolving polymer in suitable carrier. When it is given in under the skin, the polymer precipitates due to the water in tissues surrounding and controls the release of drug. The polymer and carrier, here are biocompatible & degradable [(3,4)].

3. **NANOEMULSION**: Nanoemulsions, also called as submicron emulsions, ultrafine emulsions and miniemulsions. A number of surfactants with diverse characteristics (ionic or non-ionic) had been used with such nanoemulsions. Most widely used among them were nonionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide) [(5)]. Nanoemulsion is made up of two non-miscible liquids, and that are heterogeneous system. Nanoemulsion is stabilized with co-surfactant and surfactant. It contains two non-miscible liquids substances. Nanoemulsion is used within time of date expire. It is thermodynamically and kinetically stable formulation [(6)]. The size range of nanometer is

$10^9$m.The term Nanoemulsion is made from two words i.e., nano or emulsion, also callednanometer [7]. Nanoemulsion contain essential oils the essential oils exceed the capability of hydrophobic drugs which are not soluble in the essential oils instance [8]. The nanoemulsion cross many types of barriers of biological. Nanoemulsions accelerate the in vivo drug delivery systemwhen nanoemulsion substances are unknown. In various practical cases the nanoemulsion is used in vivo experiment as vehicles for drug delivery [9]. Nanoemulsion is used in the tumor and cancer diseases. Decreasing of the interfacial tension and aqueous medium stability is higher; these both are because of only amphiphilic molecules present in the surface of oil drops. The stabilization of hydrophobic oil droplet with the help of surfactant when preparation of nanoemulsion. In hydrophobic medicament system, the nanoemulsion is used. Nanoemulsion withholding the tumor effect [10,11]. Nanoemulsions have stability against creaming, flocculation, coalescence, sedimentation. It is safe approved by the food drug administration. It is nonirritant and nontoxic use, generally recognized as safe [12,13]. Nanoemulsion consists of drug like hydrophilic and lipophilic. Nanoemulsions are stabile in nature. It is easy to formulate. Rate of absorption is high in nanoemulsion [14]. In recently nanoemulsion is used in various types of formulation such as cosmetics, antibiotics, injection and it is given by different types of routes [15]. The interfacial tension over comes adding of emulsifiers, emulsifiers' droplet size is micro [16]. When medicament is integrated in preparation, exceed the thermodynamic via the dermis. Nanoemulsion improves passage of medicament by dermis [17]. Nanoemulsion is also called ultrafine or miniemulsions. It is sized between 20200nm with narrow size distribution. Nanoemulsion is a disequilibrium arrangement of multiform collided diffusion [18]. Nanoemulsion consist pseudo-ternary structure occupying on the conversion of system of nanoemulsion administration [19] Nano Particles help to exceed range of selectivity. It is a system of colloidal particles. The solid sphere transporter along with amorphous or lipophilic exterior part carries an adverse gripe [20]. Nanoemulsion is assembled easily at intervals and automatically, it is commonly outward high energy input. Nanoemulsion is translucent [21]. Nanoemulsion is diffusion of nanoscale particle prepared by shear-induced division [22]. The diagram of nanoemulsion is break due to compression force. Nanoemulsion is used for various types of administration way. Conversion of nanoemulsion preparation by a viscous formation to the tablet solid formation. That is only case of compression force applied, which breakage the nanoemulsion system. So that is difficult to formulate the nanoemulsion into form of tablet. It is

a complicated process [23]. Nanoemulsion applicable high shear stress it is commonly attained over micro fluid and ultrasonic path commonly applied to overcome the nanoscale droplet size. Nanoemulsion are classify in multiform colloidal system, it is generally classified as their transparency and constancy [24,25]. the natural oils are used in nanoemulsion to assure substance from rust. It is fine technique using of natural oils. it is favorable drug delivery system [26] nanoemulsion show exclusive asset or properties, like as surface area is larger than size of drop is decrease, it develops dissolution rate and solubility and exceed absorption of drug from the G.I.T through the surface-active agent convinced mucosal permeability conversion [27]. Nanoemulsions also form absorption of plasma or exceed medicament bioavailability higher recreate [28]. Nanoemulsions are generally counted to be metastable systems. Indeed, the system's energy is utilized to release the surface free energy. It exceeds the interfacial area of both phases and at last single phase into another phase [29] nanoemulsion is active medicament administration implement this is only because of higher solubilizing drug substance, stability for long duration of period, and effective emulsification. Vast applications have been discovered for oral medicament administered system, to exceeding for lipophilic drugs in their bioavailability and solubility [30]. These include sustained release of the drug applied to the cornea, high penetration in the deeper layers of the ocular structure, and aqueous humor as well as ease of sterilization. Thus, these systems can achieve therapeutic action with a smaller dose and fewer systemic and ocular side effects.

**Nanoemulsion Advantages:**

1. Nanoemulsions properties are process independent, as it is a thermodynamically stable system, leading to self-emulsification.
2. Nanoemulsions carry both lipophilic and hydrophilic compounds.
3. Nanoemulsions are active transport system.
4. Easley administered.
5. Rate of absorption is increase.
6. Nanoemulsion is administered by different types of routes such as: intravenous, oral,topical ocular etc.
7. Penetration power is very rapid.
8. Energy appropriate in fewer numbers.
9. Nanoemulsion aid masking of taste.
10. Nanoemulsion is nonirritant and non-toxic.

**Nanoemulsion Disadvantages:**

**1.** Environmental parameter such as pH and temperature impact the nanoemulsion stability.

**2.** Co-surfactants and surfactants are use in bulk, to maintain the nanoemulsion stability.

**3.** The substances which have high melting point limited capacity for solubilizing **(31).**

**EYE:** Structure of the eye:

An eye is a spherical structure, protected and surrounded by eyelids eyelashes, eye brows, the lacrimal (tear-producing) apparatus and extrinsic eye muscles.

An eyeball: - An eyeball is 2.5mm in diameter, of which only 1/6th part is exposed called as anterior and remaining part is called as posterior and remains unexposed.

The eyeball is composed of three layers:

1 Fibrous Tunic or outer layer

2 Vascular Tunic or middle layer

3 Retina or innermost layer

1. Fibrous Tunic: Anterior cornea and posterior sclera composes together to form this superficial layer. Composed of fiber tissue, this layer's mechanism is to protect the eye. The curved structure of the iris controls the pupil's size, which then regulates the light intensity, reaches the retina. Its outer surface consists of no keratinized stratified squamous epithelium and a transparent layer called cornea covers iris.

2. Vascular Tunic: It is the middle layer of the eyeball and is also called as uvea and composed of three parts: choroid, ciliary body, and iris.

I. Choroid: Choroid nourishes the iris and oxygenates the photoreceptors of the retina and is located at the posterior of the eye.

II. Ciliary body: ciliary are the smooth muscles placed between sclera and the retina and maintains humidity by the secretion of aqueous humor between the medium.

III. Iris provides color to the eye, fixating pupil at its center, it helps is contraction and expanding the size of pupil.

3. Retina: The innermost layer of the eye's posterior three quarters and this is where from visual pathway begins. Retina is a photoreceptor neural tissue composed of bipolar cells, ganglion cells, equipped with color detailing function, night and peripheral vision.

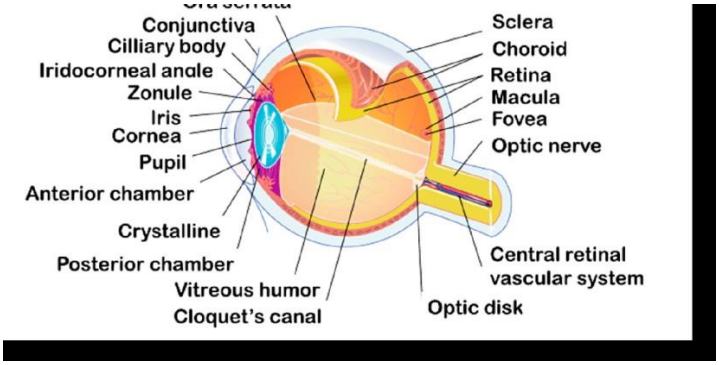



**Fig:2 Barriers of Ocular System**

Inside of an eye: Inside of an eye are these compounds:

1) The crystalline
2) The aqueous humor
3) The vitreous humor

1) The crystalline is a see-through biconvex lens and is placed between the aqueous humor and vitreous humor. Placed before iris it controls the intensity of light falling on the eye via contraction.

2) Aqueous humor is an aqueous fluid (99% water) with low mucosity; its secretion is controlled by ciliary as it is composed of ciliary body. Along with keeping the medium moist it also nourishes cornea, iris and crystalline. It gives the convex shape to the lens.

3) Vitreous humor, a transparent and high viscous fluid, contains 90% of the complete volume of an eye. It is placed between crystalline and retina.

**Blood-Ocular Barriers:**

Advances in any ocular dosage also require the administered dosage to permeate into the bloodstream at the posterior segment of an eye for an effective and diverse treatment impact along with extended reach and bioavailability. But this cannot be achieved smoothly due to the barriers at the retina, which prevents the permeation of drug into the bloodstream.

Some such classified barriers are: -

The blood retinal barrier as inner barrier in endothelial membrane: - The barrier composed of thickly packed junctions with zero leakage, which controls the fluid permeability.

An outer barrier in the retinal pigment epithelium: again, forming the tight junction with capillary endothelial cells forms a nonporous membrane made up of ciliary cells secreting aqueous humorpreventing many drug molecules into the medium [32].

## LITERATURE REVIEW

**Mowafaq M. Ghareeb (2019**): The review categorized EC in class IV of Biopharmaceutical categorization system. To get the above analysis, hydrogel of econazole nitrate, a topical nanoemulsion based gel with wide-spectrum of antimycotic application. This advised the use of nanotechnology to enhance the solubility and permeability of the drug. (6)

**Ali A and Ahmad U, Mohammed H. Taleb, Nourtan F. Abdeltawab et al (2018):** This study carried out by Ali A and Ahmad U was is in regards to the use of nanoemulsions in the manufacturing cosmetic products, considering non-polar active compound solubilization capacity of nanoemulsions and also discussed its application transdermal drug delivery system. Mohammed H. Taleb & Nourtan worked to achieve an efficient antimicrobial effect with the formulation of EO, which was tested against acne-causing bacteria, using nanoemulsion application, amplified solubility, hydrophobic nature, for EOs, oregano was formulated. They used acne mouse model to figure out the healing and antimicrobial activity of formulated EO. (7)

**Ekta Kadakia, Lipa Shah, et al (2018):** This study was focused on the interaction of nanoparticles with cellular membranes on their size and composition, reducing the size of emulsion particles to nano sizes, i.e., water to oil phase or oil to water phase along with surfactantincreased the permeation across biological barriers. (9)

**K. Gurpreet and S. K. Singh et al (2018):** This review was entirely carried on methods to prepare the nanoemulsions, via:

a) Persuasion method.
b) Brute force method.

Also, K. Gurpreet and S. K. Singh et.al examined nanoemulsions for surface tension, percent transmittance, shelf life, pH and osmolarity, viscosity, refractive index, vitro drug release etc. identifying unique characterization of nano emulsions. (5)

**Gulzar Ahmad, Florence Gattacecca et al (2018):** This study was focused on the hydrophobic drug delivery system using nanoemulsions. This was a specific study targeting the effectiveness, enhanced delivery at tumor sites, while using stable hydrophobic tween 80 oil emulsions. This study examined DHA-SBT-1214 on cancer stem cells PPT2 human prostate tumor-bearing mice. (10)

**Marion Dubald , Sandrine Bourgeois et al (2018):** This study generally covered the ocular means of the dosage, limitations, accept age ration by patients, advantages and limitations of drug delivery approach and concluded as "any topical administration method is effective on anterior part of eye thus restricted to anterior eye only, again a major drug loss happens due to tear production and ocular barriers.(3)

**Jyotsana Suyal, Maurice O. Iwunze (2017):** this analyzed the nanoemulsion application in the treatment of diseases like cancer, specific target area treatment, and nanoemulsifying and transdermal drug delivery system. Maurice's review was based on physicochemical properties of the nanoemulsions, he determined and documented refractive index, viscosity, density, pH., dielectric constant, etc, and their interpretation. (12)

**Pranita Savardekar and Amrita Bajaj (2016):** this developed nanoemulsions as an advanced form of nanoparticles and wrote of their applications in the cosmetic industry, therapy treatments, biotechnologies, preparation techniques, submicron sized emulsion application in chemotherapy & cosmetics. (31)

**Ankur Gupta, et al (2016):** This analyzed, high pressure homogenization method, bubble burst method, inversion methods, for the preparation of nanoemulsions. It also tested for the, droplet size and stability, effectiveness and effect on stability after adding various additives. It mentioned nanoemulsion as of transparent, small size, high surface area per unit volume with robust stability. (16)

**Padmadevi Chellapa1, Aref T. Mohamed (2016):** This review carried out the comparison of nano emulsion with that of liposomes and polymeric nanoparticles. The comparison included low-cost preparation method of nanoemulsion, then that of liposomes with high preparation cost, shelf life. This reviewed nano emulgel, prepared from nanoemulsion and hydrogel matrix, as bio friendly, excellent shelf life, non-satiny, soluble, removable, spreadable and agreeable appearance. (17)

**Bhavna Laxman Chauhan, Vijaya Avadhut Patil et al: (2015)** this analyzed based on the ocular capacity of tretinoin nanoemulsion as topical drug delivery method. Determined with better skin retention while testing through pig skin's Franz diffusion cells, it exhibits an improved effectiveness to *Staphylococcus epidermidis*. Its size ranges from 20-200 nm. (18)

**HongWu Sun, Chao Wei et al (2015):** In this founded out that nanoemulsions behave as an active adjuvant for respiratory syncytial virus, influenza and also active against anti-fungal, microbial & viral activity. It also poses a broad-spectrum powerful response to mucosal immune infections. (19)

**Manjit Jaiswal, Rupesh Dudhe et al (2015):** This studied the nanoemulsion based treatment method for enzyme replacement therapy in the liver, treatment of cancer, vaccination and infection of the reticuloendothelial system (RES). (20)

**Raj Kumar Mishra, G. C. Soni :( 2014)** This review covered the advantages of nanoemulsions in transdermal drug delivery mechanism and increasing the flux through skin to diffuse it into systemic circulation via designing the drug. This tried to find out the potential of the delivery mechanism of pure phytopharmaceuticals and poorly soluble drugs, which resulted in minimal side effects, high patient compliance, enhanced bioavailability by maintaining the plasma drug levels for longer time period. (21)

**Rodina Sadr Ghotbi, Marziyeh Khatibzadeh et al (2014):** this is developed the study they tried to prepare a nanoemulsion using Neem (*Azadirachtaindica*) seed with tween 20 as surfactant and used ultrasonic waves to achieve the nano droplet size. (22)

**Heni rachmawati1, chew wei yee, et a1 (2014):** They developed the Curcumin nanoemulsion tablet (125.7± 1.029 nm particle size). The nanoemulsion with a ratio of 1:8:1, which was prepared with mild stirring in oil phase using glycerol monooleate, cremophor 40, a surfactant and polyethylene glycol 400 as co-surfactant, which later was developed as a tablet using the wet granulation method. (23)

**Hiren C. Patel Ghanshyam Parmar et al (2013):** This review was based on the increased bioavailability of Ketoconazole drug via formulating an optimal nanoemulsion using Coconut as oil phase and tween 80 as co-surfactants, which was tested against the thermodynamic stability, dispersibility. The optimized product from above was found out to be with higher drug release, optimum globule size, minimum polydispersity value, lower viscosity, and overall lower surfactant concentration and co-surfactant, concluding an improved bioavailability of poorly water-soluble drugs, using the nanoemulsions. (24)

**Dale S. Aldrich (2013):** This research work was focused on the quality tests on ophthalmic preparations named as Quality Tests 771, addressing dissolution of drug, drug release and other quality tests for other all dosage forms for eye. (1)

**Ashaben Patel, Kishore Cholkar et al (2013):** This study was aimed at finding the ocular drug delivery challenges, topical drug administration barriers as various precorneal, dynamic and static ocular barriers. It spoke of advances towards developing a novel, safe and patient compliant formulation and drug delivery devices/techniques, which may surpass these barriers and maintain drug levels in tissues. (2)

**Ling Zhao, Yumeng Wei et al (2013):** This article developed of poor water solubility and low oral bioavailability of some drugs in nanoemulsions, e.g., i.e., cyclosporine (Neoral®, Gengraf®), saquinavir (Fortovase®), ritonavir (Norvir®), are approved by the US Food and Drug Administration despite of their poor water solubility. This study also developed a nanoemulsion formulation enhancing oral absorption and bioavailability of baicalin as BAN-1, BAN-2.which was later inspected for physicochemical properties and pharmacokinetics. (27)

**Ranjit Kumar Harwansh and Kartik Chandra Patra et al (2011):** This study was focused on transdermal method of glycyrrhizin (GZ), with improved permeation, stability, using minimum surfactant and cosurfactant. Based on this, we used soyabean as oil, span 80, brij 35, as surfactant and isopropyl alcohol as co surfactant to formulate nanoemulsion, which later were tested for stability, pH., droplet size, and viscosity using electron transmission microscopy. Permeation profile was determined using the Franz diffusion cells and result showed a significant increase in permeability parameters i.e., permeability coefficients & steady state flux, consisting 1% wt/wt of mono-ammonium glycyrrhizinate (MAG), 32.4% Span 80, 3.7% Brij 35, 10% isopropyl alcohol,46.5% soyabean oil and 6.4% distilled water. (28)

**Thomas Delmas, Helene Piraux (2010):** They described nanoemulsion as thermodynamically unstable system, and a system posing stable kinetic energy for long term. Moreover, this article spoke of practical and theoretical considerations, while formulation nanoemulsions via ultrasonic methods as:

a) Ostwald ripening remains as main destabilizing mechanism.

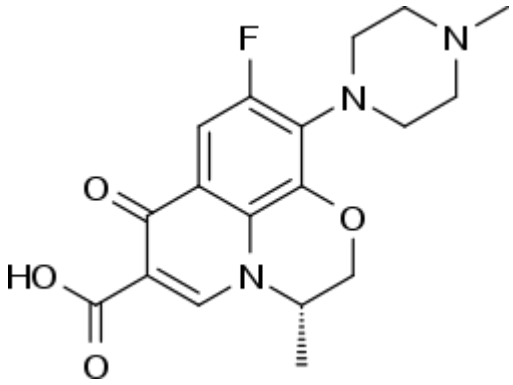
b) Nanometric size of droplets prevents coalescence.

c) Mean diameter size evolution as a function of sonication energy provides us unique curve.

d) Trapped species counteract Ostwald ripening. (29)

**Adnan Azeem, Mohammad Rizwan et al (2009):** They developed ropinirole as a model lipophilic drug to provide the screening report to help and select oil, surfactants and Cosurfactants for nanoemulsion formulation. Optimum nanoemulsion formulation can be achieved viaconsidering effective surfactant and cosurfactant mass ratio. (30)

**DRUG REVIEW:**

| | |
|---|---|
| IUPAC NAME | (-)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazinyl)-7-oxo-7H-pyrido[1,2,3-de] benzoxazine-6-carboxylic acid Hemihydrates. |
| Structure |  |
| Chemical Formula<br><br>Molecular weight | $C_{18}H_{20}FN_3O_4$<br><br>361.368g/mol g˙mol$^{-1}$ |
| Category | Antibiotic |
| Drug Class | Fluoroquinolone |
| Half-Life | 6-9 hours |
| Bioavailability | 99% |
| Trade names | Levaquin, Tavanic, Iquix |
| Binding of protein | 31% |

| | |
|---|---|
| Metabolism | N-oxide metabolites and <5% dimethyl |
| Excretion | Renal, mostly unchanged (83%) |
| Route of Administration | eye drops, IV, by mouth |
| Melting Point | 220-225°C |

**Uses:** levofloxacin belongs to the fluoroquinolone (flor-o-kwin-o lines). Group of antibiotics andit used to treatment of skin disease for e.g. Prostate, bladder, kidney and sinus. It cures the bodybacterial infection.

**Therapeutic uses:** it is used to cure the tuberculosis, diarrhea, endocarditis, anthrax, urinary tract prostate, respiratory tract, plague or can be administered by ocular system.

**Mechanism of action:** Levofloxacin is effective across the Gram-positive bacteria and Gram-negative bacteria. Such that topoisomerase IV and DNA gyrase impending by function of quinolones. To isolate the replicated DNA, Topoisomerase IV is required point to division of cell in bacteria and if no more isolated, then it stops the whole process. DNA gyrase helps in great coiling of DNA, it makes to able new cell prepared. Levofloxacin acts as a bactericide [33].

## AIM & OBJECTIVES

Design formulation development and evaluation of novel Nanoemulsion for ocular drug delivery of Levofloxacin.

1. To formulate and optimize levofloxacin loaded uncoated nanoemulsion and chitosan coated nanoemulsion for ocular delivery.
2. To characterize the developed formulations (*in vitro)*
3. To establish the mucoadhesive profile and transcorneal permeability potential of the developed systems
4. To evaluate the pharmacodynamic activity (*in vitro*) and pharmacokinetic activity (*in vivo*) ofthe developed systems

## PLAN OF WORK

The research planned and carried out in the following manner.

1. Literature Survey
2. Preformulation studies
    a. Purity and Identification studies of levofloxacin
    b. Determination of $\lambda_{max}$
    c. FT-IR spectroscopy
    d. Melting point
    e. Solubility
    f. Partition coefficient
3. Development of analytical method by UV-spectrophotometer & HPLC
    a. Standard curve of Levofloxacin in methanol
    b. Preparation of standard curve in Simulated Tear Fluid
    c. Development of analytical method by HPLC
4. Screening of excipients i.e., Oil & Smix (Surfactant & Cosurfactant mixture)
5. Phase behavior and optimization of nanoemulsion
    a. Construction of pseudoternary phase diagram.
6. Optimization of Formulation using DOE
7. Fitting the model to data
8. Influence of formulation variables on the response (CQAs)

a. Response 1: Globule Size

b. Response R2: Polydispersity Index

c. Checkpoint analysis

9. Development of Nanoemulsion

10. *In vitro* Characterization of Nanoemulsion

a. Morphology

b. Globule Size and Polydispersity Index Determination

c. Zeta potential

d. Determination of pH

e. Viscosity Measurement

f. Osmolarity

g. Refractive Index

h. In Vitro drug release study and Release Kinetics

i. Mucoadhesion strength studies

j. *Ex Vivo* transcorneal permeation study

k. Antimicrobial Efficacy

l. *In Vivo* studies

m. Ocular Irritation studies

n. Pharmacokinetics studies

o. Stability Study

## MATERIALS REQUIRED

**Table:** Company name and Chemicals used for the experiments

| S. No. | Chemical Used | Company |
|---|---|---|
| 1. | Acetic Acid | Finer Reagent |
| 2. | Levofloxacin | MMG Healthcare Ltd. Sirmour (H.P.) |
| 3. | Castor Oil | SDFCL |
| 4. | Olive Oil | HI Media |
| 5. | Dialysis Membrane | HI Media |
| 6. | Ethyl Oleate (Oleic-Acid) | CDH |
| 7. | Methanol (HPLC Grade) | SDFCL |
| 8. | n-Octanol | CDH |
| 9. | Tween 80 | SDFCL |
| 10. | Sodium Dihydrogen Phosphate | HI media |
| 11. | Soya lecithin | CDH |
| 12. | Methanol (AR) | SDFCL |
| 13. | Potassium bromide | SDFCL |
| 14. | Sodium chloride | SDFCL |
| 15. | Calcium chloride | SDFCL |
| 16. | Sodium bicarbonate | SDFCL |

| | | |
|---|---|---|
| 17. | Acetonitrile | SDFCL |
| 18. | Triethylamine | SDFCL |

**Table: List of instruments**

| **Instrument** | **Model** | **Manufacturer** |
|---|---|---|
| Bath Sonicator | 40050 | Steryl media equip system |
| Centrifuge | R-24C | Remi Instruments division Maharashtra, India |
| Digital Melting Point Apparatus | DB- 31354 | Decibels Instruments, India |
| Digital weighing balance | AB265-S/ FACT | Mettler Toledo, Switzerland |
| FT-IR Spectrophotometer | Nicolet -380 | Thermo, USA |
| Franz Diffusion cell | 4G- 01- 00-09-05 | Prefit, India |
| HPLC | 2489 | Waters |
| Hot Air Oven | PT 400 | Perfit, India |
| Melting Point Apparatus | DB- 31354 | Decibels Instruments, India |
| Magnetic stirrer | RQ-122 | Remi work, India |
| Purified water system | Milli-Q | Millipore, USA |
| Particle size analyzer | DLS 4 C | Beckman Coulter, USA |
| Shaking incubator | LSB- 1005RE | Daihan Labtech. Co. Ltd., Korea |

| | | |
|---|---|---|
| Transmission Electron Microscopy | | Hitachi |

## TENTATIVE CHAPTER PLAN

| Sr. No. | Name of Chapter |
|---|---|
| **1.** | Introduction |
| **2.** | Literature survey |
| **3.** | Research |
| **4.** | Experimental |
| **5.** | Results and Discussion |
| **6.** | Summary and Conclusion |

## PREPARATION METHODS OF NANOEMULSION

### 1. High pressure Homogenization

High pressure homogenization or piston homogenizer equipped with hydraulic shear, strong instability, or cogitation is used to produce the very narrowness nanoemulsion particle (up to 1 nm) and the process is repeated to obtain the nano droplet size & polydispersity index. The dispersed phase is diluted for high volume of fraction then emulsion is prepared thereafter. Although, preparation at high volume fractions creates coalescence. Hence, we add more surfactants to eliminate re-coalescence. To achieve smaller droplets, surfactant is diffuse in the disperse phase alternatively the continuous phase as it intensifies process, particularly the emulsions with highly viscous disperse phase and assimilation generally form of microemulsion oil in water type coincide along monolayer surfactant dramatic specific curve and balance oil. Polyethoxylated surfactant turns lipophilic on heating and as the temperature rises, the basic o/w emulsion changes to w/o emulsion forming negative curvature on surfactant monolayer. This heating involved in the process may lead to instability while administering Peptides and tretinoin. That is thermolabile drugs.

### 2. Microfluidization Method:

This method studied for mixing and with micro fluidizer equipment that enforces substance to pass by micro channel's impingement area of the interaction chamber resulting in the particles of submicron range. The coarse emulsion processed in inline homogenizer is introduced into a micro fluidizer, where it is cross by micro fluidizer chamber frequently to obtain a stable and desired size nanoemulsion. Uniformity is obtained by filtering the bulk emulsion under nitrogen, removing large droplets.

**3. Inversion of phase temperature Method:**

The inversion of phase temperature method mechanism exhibited a relation among small size of drops or replete dissolution in microemulsion in the oil. Emulsion's phase inversion are categorized as: transitional inversion initiated via changing factors, ultimately affecting the hydrophilic lipophilic balance, In Phase inversion temperature (PIT) treatment solubility of nonionic surfactants depends upon temperature. E.g., polyethoxylated surfactants, on heating it converts in the form of lipophilic owing to Polyoxyethyelene groups is too dehydrated. Which also mode fabrication of nanoemulsion applying this mechanism. It is a spontaneous process, unstablethermodynamically and formulated spontaneous nanoemulsion. In this method nonionic surfactant, water and oil In the Phase inversion method, at the room temperature non-ionic surfactants, oil, water are blended stable. Nanoemulsions prepared using the spontaneous nano emulsification processes are thermodynamically unstable.

**4. Displacement of solvent Technique:**

Displacement of solvent technique applied for nanoparticle polymer that is appropriate for the nano precipitation and this method is uninhibited nanoemulsion fabrication. The water mixable solvent for e.g., ethyl methyl ketone, ethanol acetone, dissolved oily phase in it. Organic state is discharge into aqueous state with surface active agent which helps in producing nanoemulsion by quick dispersion of organic solvent.

**5. Inversion of phase combination technique (Self nano emulsification Method)**

The inversion of phase combination technique is interesting among scientists in various pharmaceutical and other sciences. It produces nanoemulsions without several organic solvent and warmth at room temperature. The droplets size is 50nm and stable kinetically and generated through extension of water into solution of surface-active agent in oil, along affable stirtalline phases around the nanoemulsion droplets**.** [(34)]**.**

## REFERENCES


1. Aldrich DS, Bach CM, Brown W, Chambers W, Fleitman J, Hunt D, Marques M, Mille Y, Mitra AK, Platzer SM. Ophthalmic preparations. US Pharmacopeia. 2013 Sep;39(5):1-21.
2. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An overview. World journal of pharmacology. 2013;2(2):47.
3. Dubald M, Bourgeois S, Andrieu V, Fessi H. Ophthalmic drug delivery systems for antibiotherapy—a review. Pharmaceutics. 2018 Jan 13;10(1):10.
4. Gibson M. Pharmaceutical preformulation and formulation. Drugs Pharm Sci. 2001;199:199.
5. Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian Journal of Pharmaceutical Sciences. 2018 Aug 31;80(5):781-9.
6. Ghareeb MM. Design and in vitro characterization of a topical nanoemulsion-enriched hydrogel of econazole nitrate. Journal of Applied Pharmaceutical Science. 2019 Feb 4;9(1):051-7.
7. Ahmad U. Nanoemulsion as a vehicle in transdermal drug delivery. archivos de medicina. 2018;3(3):15.
8. Taleb MH, Abdeltawab NF, Shamma RN, Abdelgayed SS, Mohamed SS, Farag MA, Ramadan MA. Origanum vulgare L. essential oil as a potential anti-acne topical nanoemulsion—In vitro and in vivo study. Molecules. 2018 Aug 28;23(9):2164.
9. Kadakia E, Shah L, Amiji MM. Mathematical modeling and experimental validation of nanoemulsion-based drug transport across cellular barriers. Pharmaceutical research. 2017 Jul;34(7):1416-27.
10. Ahmad G, Gattacecca F, El Sadda R, Botchkina G, Ojima I, Egan J, Amiji M. Biodistribution and pharmacokinetic evaluations of a novel taxoid DHA-SBT-1214 in an oil-in-water nanoemulsion formulation in naive and tumor-bearing mice. Pharmaceutical research. 2018 Apr;35(4):1-3.
11. Simonazzi A, Cid AG, Villegas M, Romero AI, Palma SD, Bermúdez JM. Nanotechnology applications in drug controlled release. InDrug targeting and stimuli sensitive drug delivery systems 2018 Jan 1 (pp. 81-116). William Andrew Publishing.
12. Suyal J. Nanoemulsion: a novel approach in various pharmaceutical applications. J Pharm Sci Bioscientific Res. 2017;7:153-7.
13. Iwunze MO. The Determination of the Physico-Chemical Properties of Nanoemulsion. Journal of Materials Science and Engineering A. 2017;7(1-2):19-24.
14. Ali A, Ansari VA, Ahmad U, Akhtar J, Jahan A. Nanoemulsion: An advanced vehicle for efficient drug delivery. Drug research. 2017 Nov;67(11):617-31.

15. Thakur N, Garg G, Sharma PK, Kumar N. Nanoemulsions: a review on various pharmaceutical application. Global Journal of Pharmacology. 2012;6(3):222-5.
16. Gupta A, Eral HB, Hatton TA, Doyle PS. Nanoemulsions: formation, properties and applications. Soft matter. 2016;12(11):2826-41.
17. Chellapa P, Mohamed AT, Keleb EI, Elmahgoubi A, Eid AM, Issa YS, Elmarzugi NA. Nanoemulsion and nanoemulgel as a topical formulation. IOSR J Pharm. 2015 Oct;5(10):43-7.
18. Chauhan BL, Patil VA, Khutle NM. international journal of universal pharmacy and bio sciences.
19. Sun H, Wei C, Liu B, Jing H, Feng Q, Tong Y, Yang Y, Yang L, Zuo Q, Zhang Y, Zou Q. Induction of systemic and mucosal immunity against methicillin-resistant Staphylococcus aureus infection by a novel nanoemulsion adjuvant vaccine. International Journal of Nanomedicine. 2015;10:7275.
20. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015 Apr;5(2):123-7.
21. Mishra RK, Soni GC, Mishra R. Nanoemulsion: a novel drug delivery tool. International Journal of Pharma Research & Review. 2014 Jul;3(7):32-43.
22. Ghotbi RS, Khatibzadeh M, Kordbacheh S. Preparation of neem seed oil nanoemulsion. InProceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications 2014 Aug 11 (pp. 11-13).
23. Rachmawati H, Yee CW, Rahma A. Formulation of tablet containing curcumin nanoemulsion. Int J Pharm Pharm Sci. 2014;6(3):115-6.
24. Patel KT, Chotai NP. Pharma science monitor. Int J Pharm Sci. 1980;93.
25. Sutradhar KB, Amin ML. Nanoemulsions: increasing possibilities in drug delivery. European Journal of Nanomedicine. 2014 Mar 1;6(1):53-.
26. Karthikeyan S, Jeeva PA, Jerobin J, Amitava M, Chandrasekaran N. Formulation and characterization of nanoemulsion coatings from Azadirachta indica. International Journal of ChemTech Research. 2012;4(4):1566-70.
27. Zhao L, Wei Y, Huang Y, He B, Zhou Y, Fu J. Nanoemulsion improves the oral bioavailability of baicalin in rats: in vitro and in vivo evaluation. International journal of nanomedicine. 2013;8:3769.
28. Harwansh RK, Patra KC, Pareta SK, Singh J, Rahman MA. Nanoemulsions as vehicles for transdermal delivery of glycyrrhizin. Brazilian Journal of Pharmaceutical Sciences. 2011;47:769-78.

29. Delmas T, Piraux H, Couffin AC, Texier I, Vinet F, Poulin P, Cates ME, Bibette J. How to prepare and stabilize very small nanoemulsions. Langmuir. 2011 Mar 1;27(5):1683-92.
30. Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, Talegaonkar S. Nanoemulsion components screening and selection: a technical note. Aaps Pharmscitech. 2009 Mar;10(1):69-76.
31. Savardekar P, Bajaj A. Nanoemulsions-a review. Inter J Res Pharm and Chem. 2016;6:312-22.
32. j. Tortora, derrickson Bryan, Anatomy and physiology edition 2014, 534.
33. **https://www.ncbi.nlm.nih.gov/**
34. Lovelyn C, Attama AA. Current state of nanoemulsions in drug delivery. Journal of Biomaterials and Nanobiotechnology. 2011 Dec 9;2(05):626.